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(54) Title: GOSSYPOL CO-CRYSTALS AND THE USE THEREOF

(57) Abstract: This invention relates to compositions comprising co-crystals of (-)- gossypol with a C_{1-8} carboxylic acid or C_{1-8} sulfonic acid which are useful as inhibitors of Bcl-2 family proteins. The invention also relates to the use of cocrystals of (-)-gossypol with a C_{1-8} carboxylic acid or C_{1-8} sulfonic acid for inducing apoptosis in cells and for sensitizing cells to the induction of apoptotic cell death.

GOSSYPOL CO-CRYSTALS AND THE USE THEREOF

BACKGROUND OF THE INVENTION

Field of the Invention

[0001] This invention is in the field of medicinal chemistry. In particular, the invention relates to compositions comprising co-crystals of (-)-gossypol with a C_{1-8} carboxylic acid or C_{1-8} sulfonic acid which are useful as inhibitors of Bcl-2 family proteins. The invention also relates to the use of co-crystals of (-)-gossypol with a C_{1-8} carboxylic acid or C_{1-8} sulfonic acid for inducing apoptosis in cells and for sensitizing cells to the induction of apoptotic cell death.

Related Art

[0002] The aggressive cancer cell phenotype is the result of a variety of genetic and epigenetic alterations leading to deregulation of intracellular signaling pathways (Ponder, *Nature 411*:336 (2001)). The commonality for all cancer cells, however, is their failure to execute an apoptotic program, and lack of appropriate apoptosis due to defects in the normal apoptosis machinery is a hallmark of cancer (Lowe *et al.*, *Carcinogenesis 21*:485 (2000)). Most of the current cancer therapies, including chemotherapeutic agents, radiation, and immunotherapy, work by indirectly inducing apoptosis in cancer cells. The inability of cancer cells to execute an apoptotic program due to defects in the normal apoptotic machinery is thus often associated with an increase in resistance to chemotherapy, radiation, or immunotherapy-induced apoptosis. Primary or acquired resistance of human cancer of different origins to current treatment protocols due to apoptosis defects is a major problem in current cancer therapy (Lowe *et al.*, *Carcinogenesis 21*:485 (2000); Nicholson, *Nature 407*:810 (2000)). Accordingly, current and future efforts towards designing and developing new molecular target-specific anticancer therapies to improve survival and quality of life of cancer patients must include strategies that

specifically target cancer cell resistance to apoptosis. In this regard, targeting crucial negative regulators that play a central role in directly inhibiting apoptosis in cancer cells represents a highly promising therapeutic strategy for new anticancer drug design.

[0003] Two classes of central negative regulators of apoptosis have been identified. The first class of regulators is the inhibitor of apoptosis proteins (IAPs) (Deveraux *et al.*, *Genes Dev. 13*:239 (1999); Salvesen *et al.*, *Nat. Rev. Mol. Cell. Biol. 3*:401 (2002)). IAP proteins potently suppress apoptosis induced by a large variety of apoptotic stimuli, including chemotherapeutic agents, radiation, and immunotherapy in cancer cells.

[0004] The second class of central negative regulators of apoptosis is the Bcl-2 family of proteins (Adams *et al.*, *Science 281*:1322 (1998); Reed, *Adv. Pharmacol. 41*:501 (1997); Reed *et al.*, *J. Cell. Biochem. 60*:23 (1996)). Bcl-2 is the founding member of the family and was first isolated as the product of an oncogene. The Bcl-2 family now includes both anti-apoptotic molecules such as Bcl-2 and Bcl-X_L and pro-apoptotic molecules such as Bax, Bak, Bid, and Bad. Bcl-2 and Bcl-X_L are overexpressed in many types of human cancer (*e.g.*, breast, prostate, colorectal, lung, *etc.*), including Non-Hodgkin's lymphoma, which is caused by a chromosomal translocation (t14, 18) that leads to overexpression of Bcl-2. This suggests that many cancer cell types depend on the elevated levels of Bcl-2 and/or Bcl-X_L to survive the other cellular derangements that simultaneously both define them as cancerous or pre-cancerous cells and cause them to attempt to execute the apoptosis pathway. Also, increased expression of Bcl-2 family proteins has been recognized as a basis for the development of resistance to cancer therapeutic drugs and radiation that act in various ways to induce cell death in tumor cells.

[0005] Bcl-2 and Bcl-X_L are thought to play a role in tumor cell migration and invasion, and therefore, metastasis. Amberger *et al.*, *Cancer Res. 58*:149 (1998); Wick *et al.*, *FEBS Lett, 440*:419 (1998); Mohanam *et al.*, *Cancer Res. 53*:4143 (1993); Pedersen *et al.*, *Cancer Res.,53*:5158 (1993). Bcl-2 family proteins appear to provide tumor cells with a mechanism for surviving in new

and non-permissive environments (*e.g.*, metastatic sites), and contribute to the organospecific pattern of clinical metastatic cancer spread. Rubio, *Lab Invest. 81*:725 (2001); Fernández *et al.*, *Cell Death Differ. 7*:350 (2000)). Anti-apoptotic proteins such as Bcl-2 and/or Bcl-X_L are also thought to regulate cell-cell interactions, for example through regulation of cell surface integrins. Reed, *Nature 387*:773 (1997); Frisch *et al.*, *Curr. Opin. Cell Biol. 9*:701 (1997); Del Bufalo *et al.*, *FASEB J. 11*:947 (1997).

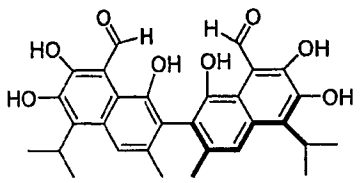
[0006] Therapeutic strategies for targeting Bcl-2 and Bcl-X_L in cancer to restore cancer cell sensitivity and overcome resistance of cancer cells to apoptosis have been extensively reviewed (Adams *et al.*, *Science 281*:1322 (1998); Reed, *Adv. Pharmacol. 41*:501 (1997); Reed *et al.*, *J. Cell. Biochem. 60*:23 (1996)). Currently, Bcl-2 antisense therapy is in several Phase III clinical trials for the treatment of solid and non-solid tumors.

[0007] Gossypol is a naturally occurring double biphenolic compound derived from crude cotton seed oil (*Gossypium sp.*). Human trials of gossypol as a male contraceptive have demonstrated the safety of long term administration of these compounds (Wu, *Drugs 38*:333 (1989)). Gossypol has more recently been shown to have some anti-proliferative effects (Flack *et al.*, *J. Clin. Endocrinol. Metab. 76*:1019 (1993); Bushunow *et al.*, J. Neuro-Oncol. 43:79, (1999); Van Poznak *et al.*, *Breast Cancer Res. Treat. 66*:239 (2001)). (-)-Gossypol and its derivatives recently have been shown to be potent inhibitors of Bcl-2 and Bcl-X_L and to have strong anti-cancer activity (U.S. Patent Application No. 2003/0008924).

[0008] A composition comprising racemic gossypol and acetic acid is known in the art (Sigma-Aldrich Corp., St. Louis, MO). Previous attempts to crystallize (-)-gossypol have resulted in crystals that are too poor for X-ray analysis (Gdaniec *et al.*, "Gossypol," in Comprehensive Supramolecular Chemistry (Atwood *et al.* eds.), Vol. 6, Pergamon) or in co-crystals of (-)-gossypol and acetone when using a solution of racemic gossypol acetic acid in acetone (Dowd *et al.*, *J. Am. Oil Chem. Soc. 76*:1343 (1999)).

SUMMARY OF THE INVENTION

[0009] The present invention relates to compositions comprising co-crystals of (-)-gossypol (formula I) with a C_{1-8} carboxylic acid or C_{1-8} sulfonic acid ("(-)-gossypol co-crystals"). These compositions are useful for inhibiting the activity of anti-apoptotic Bcl-2 family proteins, inducing apoptosis in cells, and increasing the sensitivity of cells to inducers of apoptosis.



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[0010] It is generally accepted that the inability of cancer cells or their supporting cells to undergo apoptosis in response to genetic lesions or exposure to inducers of apoptosis (such as anticancer agents and radiation) is a major factor in the onset and progression of cancer. The induction of apoptosis in cancer cells or their supporting cells (*e.g.*, neovascular cells in the tumor vasculature) is thought to be a universal mechanism of action for virtually all of the effective cancer therapeutic drugs or radiation therapies on the market or in practice today. One reason for the inability of a cell to undergo apoptosis is increased expression and accumulation of anti-apoptotic Bcl-2 family proteins.

[0011] The present invention contemplates that exposure of animals suffering from cancer to therapeutically effective amounts of (-)-gossypol co-crystal that inhibit the function(s) of anti-apoptotic Bcl-2 family proteins will kill cancer cells or supporting cells outright (those cells whose continued survival is dependent on the overactivity of Bcl-2 family proteins) and/or render such

cells as a population more susceptible to the cell death-inducing activity of cancer therapeutic drugs or radiation therapies. The present invention contemplates that (-)-gossypol co-crystals will satisfy an unmet need for the treatment of multiple cancer types, either when administered as monotherapy to induce apoptosis in cancer cells dependent on anti-apoptotic Bcl-2 family proteins function, or when administered in a temporal relationship with other cell death-inducing cancer therapeutic drugs or radiation therapies so as to render a greater proportion of the cancer cells or supportive cells susceptible to executing the apoptosis program compared to the corresponding proportion of cells in an animal treated only with the cancer therapeutic drug or radiation therapy alone.

[0012] In certain embodiments of the invention, it is expected that combination treatment of animals with a therapeutically effective amount of a composition of the present invention and a course of an anticancer agent or radiation will produce a greater tumor response and clinical benefit in such animals compared to those treated with the composition or anticancer drugs/radiation alone. Put another way, because the compositions lower the apoptotic threshold of all cells that express anti-apoptotic Bcl-2 family proteins, the proportion of cells that successfully execute the apoptosis program in response to the apoptosis inducing activity of anticancer drugs/radiation will be increased. Alternatively, the compositions of the present invention are expected to allow administration of a lower, and therefore less toxic and more tolerable, dose of an anticancer agent and/or radiation to produce the same tumor response/clinical benefit as the conventional dose of the anticancer agent/radiation alone. Since the doses for all approved anticancer drugs and radiation treatments are known, the present invention contemplates combination therapies with various combinations of known drugs/treatments with the present compositions. Also, since the compositions of the present invention act at least in part by inhibiting anti-apoptotic Bcl-2 family proteins, the exposure of cancer cells and supporting cells to therapeutically effective amounts of the compositions can be

temporally linked to coincide with the attempts of cells to execute the apoptosis program in response to the anticancer agent or radiation therapy. Thus, in some embodiments, administering the compositions of the present invention in connection with certain temporal relationships, will provide especially efficacious therapeutic practices.

[0013] (-)-Gossypol co-crystal is useful for the treatment, amelioration, or prevention of disorders responsive to induction of apoptotic cell death, *e.g.*, disorders characterized by dysregulation of apoptosis, including hyperproliferative diseases such as cancer. In certain embodiments, (-)-gossypol co-crystal can be used to treat, ameliorate, or prevent cancer that is characterized by resistance to cancer therapies (*e.g.*, those which are chemoresistant, radiation resistant, hormone resistant, and the like). In additional embodiments, (-)-gossypol co-crystal can be used to treat, ameliorate, or prevent metastatic cancer. In other embodiments, (-)-gossypol co-crystal can be used to treat hyperproliferative diseases characterized by overexpression of anti-apoptotic Bcl-2 family proteins.

[0014] The present invention provides methods of treating a viral, microbial, or parasitic infection in an animal, comprising administering to said animal a therapeutically effective amount of (-)-gossypol co-crystal.

[0015] The present invention provides pharmaceutical compositions comprising (-)-gossypol co-crystal and a pharmaceutically acceptable carrier.

[0016] The invention further provides methods of making a pharmaceutical composition comprising admixing (-)-gossypol co-crystal in a therapeutically effective amount to induce apoptosis in cells or to sensitize cells to inducers of apoptosis with a pharmaceutically acceptable carrier

[0017] The invention further provides kits comprising (-)-gossypol co-crystal and instructions for administering the composition to an animal. The kits may optionally contain other therapeutic agents, *e.g.*, anticancer agents.

[0018] The invention also provides methods of making (-)-gossypol co-crystal. For example, co-crystals may be prepared by a method comprising dissolving (-)-gossypol in acetone to form a solution, filtering the solution,

adding a C_{1-8} carboxylic acid or C_{1-8} sulfonic acid into the solution with mixing until the solution turns turbid, leaving the turbid solution at room temperature then at a reduced temperature to form co-crystals, collecting the co-crystals, washing the co-crystals with a solvent, and drying the co-crystals.

BRIEF DESCRIPTION OF THE DRAWINGS/FIGURES

[0019] Figure 1 shows the ^{1}H NMR spectrum of (-)-gossypol acetic acid co-crystal.

[0020] Figure 2 shows the ^{13}C NMR spectrum of (-)-gossypol acetic acid co-crystal.

[0021] Figure 3 shows the infrared spectrum of (-)-gossypol acetic acid co-crystal.

[0022] Figure 4 shows the mass spectrum of (-)-gossypol acetic acid co-crystal.

[0023] Figure 5 shows the X-ray powder diffraction spectrum of (-)-gossypol acetic acid co-crystal.

DETAILED DESCRIPTION OF THE INVENTION

[0024] The present invention relates to compositions comprising co-crystals of (-)-gossypol with a C_{1-8} carboxylic acid or C_{1-8} sulfonic acid ("(-)-gossypol co-crystals"), which are useful as inhibitors of anti-apoptotic Bcl-2 family proteins. By inhibiting anti-apoptotic Bcl-2 family proteins, the (-)-gossypol sensitizes cells to inducers of apoptosis and, in some instances, itself induces apoptosis. Therefore, the invention relates to methods of sensitizing cells to inducers of apoptosis and to methods of inducing apoptosis in cells, comprising administering (-)-gossypol co-crystal alone or in combination with an inducer of apoptosis. The invention further relates to methods of treating, ameliorating, or preventing disorders in an animal that are responsive to induction of apoptosis comprising administering to the animal (-)-gossypol co-

crystal and an inducer of apoptosis. Such disorders include those characterized by a dysregulation of apoptosis and those characterized by overexpression of anti-apoptotic Bcl-2 family proteins.

[0025] The terms "(-)-gossypol," or "(-)-gossypol compound/composition," as used herein, refer to an optically active composition of gossypol wherein the active molecules comprising the composition rotate plane polarized light counterclockwise (*e.g.*, levorotatory molecules) as measured by a polarimeter. Preferably, the (-)-gossypol compound has an enantiomeric excess of 1% to 100%. In one embodiment, the (-)-gossypol compound has an enantiomeric excess of at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% (-)-gossypol. In one example of a "(-)-gossypol compound", the specific rotation ($[\alpha]_D$) of the compound is about -350° to about -390°, about -375° to about -390°, or about -385° to about -390°. (*See e.g.*, Dowd, Chirality, 15:486 (2003); Ciesielska *et al.*, Chem. Phys. Lett. 353:69 (2992); Freedman *et al.*, Chirality, 15:196 (2003); and Zhou *et al.*, Kexue Tongbao, 28:1574 (1983)). Methods for resolving racemic gossypol compounds into substantially purified (+)- or (-)-gossypol are known (*See e.g.*, Zhou *et al.*, Kexue Tongbao, 28:1574 (1983) (wherein: *L*-phenylalanine methyl ester was mixed with the aldehyde groups of gossypol to form a Schiff's base with two diastereoisomers which were then resolved on a normal silica flash chromatography column. The filtrate was concentrated, and the residue was purified by chromatography on silica gel eluting with hexanes:EtOAc=3:1 to give two fractions. Acid hydrolysis of the two fractions in 5N HCl:THF (1:5, room temperature, overnight) regenerated the individual gossypol enantiomers, respectively. The first fraction with a higher R_f value contained (-)-gossypol, and the second fraction with a lower R_f value contained (+)-gossypol. The crude gossypol fractions were extracted into ether from the residue after removing THF from the reaction mixture. The gossypol fractions were then purified by chromatography on silica gel and eluted with hexanes:EtOAc (3:1 ratio) to give optically pure gossypol, with a yield of 30-40% in two steps. The optical rotatory dispersion values for these

products were α_D= -352° (c=0.65, $CHCl_3$) for (-)-gossypol, and α_D= +341° (c= 0.53, $CHCl_3$)).

[0026] The term "C_{1-8} carboxylic acid," as used herein, refers to straight-chained or branched, aromatic or non-aromatic, saturated or unsaturated, substituted or unsubstituted C_{1-8} carboxylic acid, including, but not limited to, formic acid, acetic acid, propionic acid, n-butyric acid, t-butyric acid, n-pentanoic acid, 2-pentanoic acid, n-hexanoic acid, 2-hexanoic acid, n-heptanoic acid, n-octanoic acid, acrylic acid, succinic acid, fumaric acid, malic acid, tartaric acid, citric acid, lactic acid, and benzoic acid.

[0027] The term "C_{1-8} sulfonic acid," as used herein, refers to straight-chained or branched, aromatic or non-aromatic, saturated or unsaturated, substituted or unsubstituted C_{1-8} sulfonic acid, including, but not limited to, methanesulfonic acid, ethanesulfonic acid, n-propanesulfonic acid, 2-propanesulfonic acid, n-butanesulfonic acid, n-pentanesulfonic acid n-hexanesulfonic acid, n-heptanesulfonic acid, n-octanesulfonic acid, and benzenesulfonic acid.

[0028] The term "(-)-gossypol co-crystal," as used herein, refers to a composition comprising co-crystals of (-)-gossypol and a C_{1-8} carboxylic acid or C_{1-8} sulfonic acid.

[0029] The term "Bcl-2 family proteins," as used herein, refers to both the anti-apoptotic members of the Bcl-2 family, including, but not limited to, Bcl-2, Bcl-XL, Mcl-1, A1/BFL-1, BOO-DIVA, Bcl-w, Bcl-6, Bcl-8, and Bcl-y, and the pro-apoptotic members of the Bcl-2 family, including, but not limited to, Bak, Bax, Bad, tBid, Hrk, Bim, Bmf, as well as other Bcl-2 homology domain 3 (BH3) containing proteins that are regulated by gossypol compounds.

[0030] The term "overexpression of anti-apoptotic Bcl-2 family proteins," as used herein, refers to an elevated level (*e.g.*, aberrant level) of mRNAs encoding for an anti-apoptotic Bcl-2 family protein(s), and/or to elevated levels of anti-apoptotic Bcl-2 family protein(s) in cells as compared to similar corresponding non-pathological cells expressing basal levels of mRNAs encoding anti-apoptotic Bcl-2 family proteins or having basal levels of anti-

apoptotic Bcl-2 family proteins. Methods for detecting the levels of mRNAs encoding anti-apoptotic Bcl-2 family proteins or levels of anti-apoptotic Bcl-2 family proteins in a cell include, but are not limited to, Western blotting using anti-apoptotic Bcl-2 family protein antibodies, immunohistochemical methods, and methods of nucleic acid amplification or direct RNA detection. As important as the absolute level of anti-apoptotic Bcl-2 family proteins in cells is to determining that they overexpress anti-apoptotic Bcl-2 family proteins, so also is the relative level of anti-apoptotic Bcl-2 family proteins to other pro-apoptotic signaling molecules (*e.g.*, pro-apoptotic Bcl-2 family proteins) within such cells. When the balance of these two are such that, were it not for the levels of the anti-apoptotic Bcl-2 family proteins, the pro-apoptotic signaling molecules would be sufficient to cause the cells to execute the apoptosis program and die, said cells would be dependent on the anti-apoptotic Bcl-2 family proteins for their survival. In such cells, exposure to an inhibiting effective amount of an anti-apoptotic Bcl-2 family protein inhibitor will be sufficient to cause the cells to execute the apoptosis program and die. Thus, the term "overexpression of an anti-apoptotic Bcl-2 family protein" also refers to cells that, due to the relative levels of pro-apoptotic signals and anti-apoptotic signals, undergo apoptosis in response to inhibiting effective amounts of compounds that inhibit the function of anti-apoptotic Bcl-2 family proteins.

[0031] The terms "anticancer agent" and "anticancer drug," as used herein, refer to any therapeutic agent (*e.g.*, chemotherapeutic compounds and/or molecular therapeutic compounds), radiation therapies, or surgical interventions, used in the treatment of hyperproliferative diseases such as cancer (*e.g.*, in mammals).

[0032] The term "therapeutically effective amount," as used herein, refers to that amount of the therapeutic agent sufficient to result in amelioration of one or more symptoms of a disorder, or prevent advancement of a disorder, or cause regression of the disorder. For example, with respect to the treatment of cancer, a therapeutically effective amount preferably refers to the amount of a

therapeutic agent that decreases the rate of tumor growth, decreases tumor mass, decreases the number of metastases, increases time to tumor progression, or increases survival time by at least 5%, preferably at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 100%.

[0033] The terms "sensitize" and "sensitizing," as used herein, refer to making, through the administration of a first agent (*e.g.*, a compound of Formula I), an animal or a cell within an animal more susceptible, or more responsive, to the biological effects (*e.g.*, promotion or retardation of an aspect of cellular function including, but not limited to, cell growth, proliferation, invasion, angiogenesis, or apoptosis) of a second agent. The sensitizing effect of a first agent on a target cell can be measured as the difference in the intended biological effect (*e.g.*, promotion or retardation of an aspect of cellular function including, but not limited to, cell growth, proliferation, invasion, angiogenesis, or apoptosis) observed upon the administration of a second agent with and without administration of the first agent. The response of the sensitized cell can be increased by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 100%, at least 150%, at least 200%, at least 350%, at least 300%, at least 350%, at least 400%, at least 450%, or at least 500% over the response in the absence of the first agent.

[0034] The term "dysregulation of apoptosis," as used herein, refers to any aberration in the ability of (*e.g.*, predisposition) a cell to undergo cell death via apoptosis. Dysregulation of apoptosis is associated with or induced by a variety of conditions, including for example, autoimmune disorders (*e.g.*, systemic lupus erythematosus, rheumatoid arthritis, graft-versus-host disease, myasthenia gravis, or Sjögren's syndrome), chronic inflammatory conditions (*e.g.*, psoriasis, asthma or Crohn's disease), hyperproliferative disorders (*e.g.*, tumors, B cell lymphomas, or T cell lymphomas), viral infections (*e.g.*, herpes,

papilloma, or HIV), and other conditions such as osteoarthritis and atherosclerosis. It should be noted that when the dysregulation is induced by or associated with a viral infection, the viral infection may or may not be detectable at the time dysregulation occurs or is observed. That is, viral-induced dysregulation can occur even after the disappearance of symptoms of viral infection.

[0035] The term "hyperproliferative disease," as used herein, refers to any condition in which a localized population of proliferating cells in an animal is not governed by the usual limitations of normal growth. Examples of hyperproliferative disorders include tumors, neoplasms, lymphomas and the like. A neoplasm is said to be benign if it does not undergo invasion or metastasis and malignant if it does either of these. A "metastatic" cell means that the cell can invade and destroy neighboring body structures. Hyperplasia is a form of cell proliferation involving an increase in cell number in a tissue or organ without significant alteration in structure or function. Metaplasia is a form of controlled cell growth in which one type of fully differentiated cell substitutes for another type of differentiated cell.

[0036] The pathological growth of activated lymphoid cells often results in an autoimmune disorder or a chronic inflammatory condition. As used herein, the term "autoimmune disorder" refers to any condition in which an organism produces antibodies or immune cells which recognize the organism's own molecules, cells or tissues. Non-limiting examples of autoimmune disorders include autoimmune hemolytic anemia, autoimmune hepatitis, Berger's disease or IgA nephropathy, celiac sprue, chronic fatigue syndrome, Crohn's disease, dermatomyositis, fibromyalgia, graft versus host disease, Grave's disease, Hashimoto's thyroiditis, idiopathic thrombocytopenia purpura, lichen planus, multiple sclerosis, myasthenia gravis, psoriasis, rheumatic fever, rheumatic arthritis, scleroderma, Sjögren's syndrome, systemic lupus erythematosus, type 1 diabetes, ulcerative colitis, vitiligo, and the like.

[0037] The term "neoplastic disease," as used herein, refers to any abnormal growth of cells being either benign (non-cancerous) or malignant (cancerous).

[0038] The term "anti-neoplastic agent," as used herein, refers to any compound that retards the proliferation, growth, or spread of a targeted (*e.g.*, malignant) neoplasm.

[0039] The terms "prevent," "preventing," and "prevention," as used herein, refer to a decrease in the occurrence of pathological cells (*e.g.*, hyperproliferative or neoplastic cells) in an animal. The prevention may be complete, *e.g.*, the total absence of pathological cells in a subject. The prevention may also be partial, such that the occurrence of pathological cells in a subject is less than that which would have occurred without the present invention.

[0040] The term "synergistic," as used herein, refers to an effect obtained when (-)-gossypol co-crystal and a second agent are administered together (*e.g.*, at the same time or one after the other) that is greater than the additive effect of (-)-gossypol co-crystal and the second agent when administered individually. The synergistic effect allows for lower doses of (-)-gossypol co-crystal and/or the second agent to be administered or provides greater efficacy at the same doses. The synergistic effect obtained can be at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 100%, at least 125%, at least 150%, at least 175%, at least 200%, at least 250%, at least 300%, at least 350%, at least 400%, or at least 500% more than the additive effect of the (-)-gossypol co-crystal compound and the second agent when administered individually. For example, with respect to the treatment of cancer, the synergistic effect can be a decrease in the rate of tumor growth, a decrease in tumor mass, a decrease in the number of metastases, an increase in time to tumor progression, or an increase in survival time. The co-administration of (-)-gossypol co-crystal and an anticancer agent may allow for the use of lower doses of (-)-gossypol co-crystal and/or the anticancer agent such that the cancer is effectively treated while avoiding any substantial toxicity to the subject.

[0041] The term "about," as used herein, includes the recited number +/- 10%. Thus, "about 0.5" means 0.45 to 0.55.

[0042] The inhibitors of anti-apoptotic Bcl-2 family proteins of the present invention are compositions comprising co-crystals of (-)-gossypol with a C_{1-8} carboxylic acid or C_{1-8} sulfonic acid ("(-)-gossypol co-crystals"). (-)-Gossypol co-crystal is expected to be more stable than (-)-gossypol alone. Those skilled in the art will appreciate the importance of compound stability in the manufacturing, storage, shipping, and/or handling of pharmaceutical compositions. The present compositions are expected to be more stable than previously described compositions comprising (-)-gossypol. Any C_{1-8} carboxylic acid or C_{1-8} sulfonic acid that is capable of stabilizing (-)-gossypol can be used in the invention. The molar ratio of (-)- gossypol to carboxylic acid or sulfonic acid in (-)-gossypol co-crystal ranges from about 10:1 to about 1:10, preferably about 2:1 to about 1:2, more preferably about 1:1. In some embodiments, the molar ratio of (-)-gossypol to carboxylic acid or sulfonic acid in (-)-gossypol co-crystal can be about 10:1, 9:1, 8:1, 7:1, 6:1, 5:1, 4:1, 3:1, 2:1, 1.5:1, 1:1, 1:1.5, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9, or 1:10.

[0043] In one embodiment of the invention the C_{1-8} carboxylic acid is acetic acid. In another embodiment, (-)-gossypol co-crystal comprises (-)-gossypol and acetic acid in a molar ratio of about 1:1. In a preferred embodiment, the 1:1 co-crystal of (-)-gossypol and acetic acid is in the form of yellow or pale yellow needle-shaped crystals. In another preferred embodiment, the co-crystal is characterized by integration of ^{1}H NMR spectrum at δ 2.11 (s, 3H) which is one methyl signal of acetic acid and δ 2.18 (s, 6H) which is two methyl signals of gossypol.

[0044] The compositions of this invention may be prepared using methods known to those of skill in the art and as disclosed in the Examples. In one embodiment, co-crystals are prepared by dissolving (-)-gossypol in acetone to form a solution, filtering the solution, adding a C_{1-8} carboxylic acid or C_{1-8} sulfonic acid into the solution with mixing until the solution turns turbid, leaving the turbid solution at room temperature and then at reduced temperature to form co-crystals, collecting the co-crystals, washing the co-crystals with a solvent, and drying the co-crystals. In one embodiment, the

solution is mixed by constant stirring. Reduced temperature is less than about 20°C, preferably about 0-15°C, more preferably about 4°C. The time for co-crystal formation may range from 1 hour to 1 day; preferably the time is about 1-4 hours. The co-crystals may be collected by any suitable means, including by filtration. The solvent for washing the co-crystals may be any suitable solvent, *e.g.*, hexane, pentane, benzene, toluene, or petroleum ether. The washed co-crystals may be dried at room temperature, preferably in a lightproof container. The co-crystals may also be dried in a vacuum drier, preferably at an elevated temperature (*e.g.*, about 30-60°C, more preferably about 40°C) for about 6-72 hours, preferably about 12-48 hours.

[0045] (-)-Gossypol has been shown to bind to Bcl-2 and Bcl-X_L at the BH3 binding groove and to have significant anticancer activity (U.S. Patent Application No. 2003/0008924). An important aspect of the present invention is that (-)-gossypol co-crystal binds to and inhibits anti-apoptotic Bcl-2 proteins in the same manner as gossypol. However, (-)-gossypol co-crystal is expected to be more stable than (-)-gossypol. Moreover, (-)-gossypol is a more potent inhibitor than racemic gossypol. Thus, compositions comprising (-)-gossypol co-crystal may be used to induce apoptosis and also potentiate the induction of apoptosis in response to apoptosis induction signals. It is contemplated that these compositions sensitize cells to inducers of apoptosis, including cells that are resistant to such inducers. The compositions of the present invention can be used to induce apoptosis in any disorder that can be treated, ameliorated, or prevented by the induction of apoptosis. Thus, the present invention provides compositions and methods for targeting animals characterized as overexpressing an anti-apoptotic Bcl-2 family protein. In some of the embodiments, the cells (*e.g.*, cancer cells) show elevated expression levels of one or more anti-apoptotic Bcl-2 family proteins as compared to non-pathological samples (*e.g.*, non-cancerous cells). In other embodiments, the cells operationally manifest elevated expression levels of anti-apoptotic Bcl-2 family proteins by virtue of executing the apoptosis program and dying in response to administration of an inhibiting effective

amount of (-)-gossypol co-crystal, said response occurring, at least in part, due to the dependence in such cells on anti-apoptotic Bcl-2 family protein function for their survival.

[0046] In some embodiments, the compositions and methods of the present invention are used to treat diseased cells, tissues, organs, or pathological conditions and/or disease states in an animal (*e.g.*, a mammalian subject including, but not limited to, humans and veterinary animals). In this regard, various diseases and pathologies are amenable to treatment or prophylaxis using the present methods and compositions. A non-limiting exemplary list of these diseases and conditions includes, but is not limited to, cancers such as breast cancer, prostate cancer, lymphoma, skin cancer, pancreatic cancer, colon cancer, melanoma, malignant melanoma, ovarian cancer, brain cancer, primary brain carcinoma, head–neck cancer, glioma, glioblastoma, liver cancer, bladder cancer, non-small cell lung cancer, head or neck carcinoma, breast carcinoma, ovarian carcinoma, lung carcinoma, small-cell lung carcinoma, Wilms' tumor, cervical carcinoma, testicular carcinoma, bladder carcinoma, pancreatic carcinoma, stomach carcinoma, colon carcinoma, prostatic carcinoma, genitourinary carcinoma, thyroid carcinoma, esophageal carcinoma, myeloma, multiple myeloma, adrenal carcinoma, renal cell carcinoma, endometrial carcinoma, adrenal cortex carcinoma, malignant pancreatic insulinoma, malignant carcinoid carcinoma, choriocarcinoma, mycosis fungoides, malignant hypercalcemia, cervical hyperplasia, leukemia, acute lymphocytic leukemia, chronic lymphocytic leukemia, acute myelogenous leukemia, chronic myelogenous leukemia, chronic granulocytic leukemia, acute granulocytic leukemia, hairy cell leukemia, neuroblastoma, rhabdomyosarcoma, Kaposi's sarcoma, polycythemia vera, essential thrombocytosis, Hodgkin's disease, non-Hodgkin's lymphoma, soft-tissue sarcoma, osteogenic sarcoma, primary macroglobulinemia, and retinoblastoma, and the like; T and B cell mediated autoimmune diseases, inflammatory diseases, infections, hyperproliferative diseases, AIDS, degenerative conditions, vascular diseases, and the like. In some

embodiments, the cancer cells being treated are metastatic. In other embodiments, the cancer cells being treated are resistant to anticancer agents.

[0047] In some embodiments, infections suitable for treatment with the compositions and methods of the present invention include, but are not limited to, infections caused by viruses, bacteria, fungi, parasites, mycoplasma, prions, and the like.

[0048] Some embodiments of the present invention provide methods for administering an effective amount of (-)-gossypol co-crystal and at least one additional therapeutic agent (including, but not limited to, chemotherapeutic agents, antineoplastic agents, antimicrobial agents, antiviral agents, antifungal agents, and anti-inflammatory agents) and/or therapeutic technique (*e.g.*, surgical intervention, and/or radiotherapies). In some embodiments, the combination of (-)-gossypol co-crystal and one or more therapeutic agents will have a greater effect as compared to the administration of either compound alone. In other embodiments, the combination of (-)-gossypol co-crystal and one or more therapeutic agents is expected to result in a synergistic effect (*i.e.*, more than additive) as compared to the administration of either one alone.

[0049] A number of suitable anticancer agents are contemplated for use in the methods of the present invention. Indeed, the present invention contemplates, but is not limited to, administration of numerous anticancer agents such as: agents that induce apoptosis; polynucleotides (*e.g.*, anti-sense, ribozymes, siRNA); polypeptides (*e.g.*, enzymes and antibodies); biological mimetics (*e.g.*, gossypol or BH3 mimetics); agents that bind (*e.g.*, oligomerize or complex) with a Bcl-2 family protein such as Bax; alkaloids; alkylating agents; antitumor antibiotics; antimetabolites; hormones; platinum compounds; monoclonal or polyclonal antibodies (*e.g.*, antibodies conjugated with anticancer drugs, toxins, defensins), toxins; radionuclides; biological response modifiers (*e.g.*, interferons (*e.g.*, IFN-α) and interleukins (*e.g.*, IL-2)); adoptive immunotherapy agents; hematopoietic growth factors; agents that induce tumor cell differentiation (*e.g.*, all-trans-retinoic acid); gene therapy reagents (*e.g.*, antisense therapy reagents and nucleotides); tumor vaccines;

angiogenesis inhibitors; proteosome inhibitors: NF-KB modulators; anti-CDK compounds; HDAC inhibitors; and the like. Numerous other examples of chemotherapeutic compounds and anticancer therapies suitable for co-administration with the disclosed compounds are known to those skilled in the art.

[0050] In preferred embodiments, anticancer agents comprise agents that induce or stimulate apoptosis. Agents that induce apoptosis include, but are not limited to, radiation (*e.g.*, X-rays, gamma rays, UV); kinase inhibitors (*e.g.*, epidermal growth factor receptor (EGFR) kinase inhibitor, vascular growth factor receptor (VGFR) kinase inhibitor, fibroblast growth factor receptor (FGFR) kinase inhibitor, platelet-derived growth factor receptor (PDGFR) kinase inhibitor, and Bcr-Abl kinase inhibitors (such as GLEEVEC)); antisense molecules; antibodies (*e.g.*, HERCEPTIN, RITUXAN, ZEVALIN, BEXXAR, and AVASTIN); anti-estrogens (*e.g.*, raloxifene and tamoxifen); anti-androgens (*e.g.*, flutamide, bicalutamide, finasteride, aminoglutethamide, ketoconazole, and corticosteroids); cyclooxygenase 2 (COX-2) inhibitors (*e.g.*, celecoxib, meloxicam, NS-398, and non-steroidal anti-inflammatory drugs); anti-inflammatory drugs (*e.g.*, butazolidin, DECADRON, DELTASONE, dexamethasone, dexamethasone intensol, DEXONE, HEXADROL, hydroxychloroquine, METICORTEN, ORADEXON, ORASONE, oxyphenbutazone, PEDIAPRED, phenylbutazone, PLAQUENIL, prednisolone, prednisone, PRELONE, and TANDEARIL); and cancer chemotherapeutic drugs (*e.g.*, irinotecan (CAMPTOSAR), CPT-11, fludarabine (FLUDARA), dacarbazine, dexamethasone, mitoxantrone, MYLOTARG, VP-16, cisplatin, carboplatin, oxaliplatin, 5-FU, doxorubicin, gemcitabine, bortezomib, gefitinib, bevacizumab, TAXOTERE or TAXOL); cellular signaling molecules; ceramides and cytokines; staurosporine, and the like.

[0051] In still other embodiments, the compositions and methods of the present invention provide (-)-gossypol co-crystal and at least one anti-hyperproliferative or antineoplastic agent; *e.g.*, selected from alkylating

agents, antimetabolites, and natural products (*e.g.*, herbs and other plant and/or animal derived compounds).

[0052] Alkylating agents suitable for use in the present compositions and methods include, but are not limited to: 1) nitrogen mustards (*e.g.*, mechlorethamine, cyclophosphamide, ifosfamide, melphalan (L-sarcolysin); and chlorambucil); 2) ethylenimines and methylmelamines (*e.g.*, hexamethylmelamine and thiotepa); 3) alkyl sulfonates (*e.g.*, busulfan); 4) nitrosoureas (*e.g.*, carmustine (BCNU); lomustine (CCNU); semustine (methyl-CCNU); and streptozocin (streptozotocin)); and 5) triazenes (*e.g.*, dacarbazine (dimethyltriazenoimid-azolecarboxamide).

[0053] In some embodiments, antimetabolites suitable for use in the present compositions and methods include, but are not limited to: 1) folic acid analogs (*e.g.*, methotrexate (amethopterin)); 2) pyrimidine analogs (*e.g.*, fluorouracil (5-fluorouracil), floxuridine (fluorode-oxyuridine), and cytarabine (cytosine arabinoside)); and 3) purine analogs (*e.g.*, mercaptopurine (6-mercaptopurine), thioguanine (6-thioguanine), and pentostatin (2'-deoxycoformycin)).

[0054] In still further embodiments, chemotherapeutic agents suitable for use in the compositions and methods of the present invention include, but are not limited to: 1) vinca alkaloids (*e.g.*, vinblastine, vincristine); 2) epipodophyllotoxins (*e.g.*, etoposide and teniposide); 3) antibiotics (*e.g.*, dactinomycin (actinomycin D), daunorubicin (daunomycin; rubidomycin), doxorubicin, bleomycin, plicamycin (mithramycin), and mitomycin (mitomycin C)); 4) enzymes (*e.g.*, L-asparaginase); 5) biological response modifiers (*e.g.*, interferon-alfa); 6) platinum coordinating complexes (*e.g.*, cisplatin and carboplatin); 7) anthracenediones (*e.g.*, mitoxantrone); 8) substituted ureas (*e.g.*, hydroxyurea); 9) methylhydrazine derivatives (*e.g.*, procarbazine (N-methylhydrazine)); 10) adrenocortical suppressants (*e.g.*, mitotane (o,p'–DDD) and aminoglutethimide); 11) adrenocorticosteroids (*e.g.*, prednisone); 12) progestins (*e.g.*, hydroxyprogesterone caproate, medroxyprogesterone acetate, and megestrol acetate); 13) estrogens (*e.g.*, diethylstilbestrol and ethinyl estradiol); 14) antiestrogens (*e.g.*, tamoxifen);

15) androgens (*e.g.*, testosterone propionate and fluoxymesterone); 16) antiandrogens (*e.g.*, flutamide): and 17) gonadotropin-releasing hormone analogs (*e.g.*, leuprolide).

[0055] Any oncolytic agent that is routinely used in a cancer therapy context finds use in the compositions and methods of the present invention. For example, the U.S. Food and Drug Administration maintains a formulary of oncolytic agents approved for use in the United States. International counterpart agencies to the U.S.F.D.A. maintain similar formularies. Table 1 provides a list of exemplary antineoplastic agents approved for use in the U.S. Those skilled in the art will appreciate that the "product labels" required on all U.S. approved chemotherapeutics describe approved indications, dosing information, toxicity data, and the like, for the exemplary agents.

Table 1

Aldesleukin (des-alanyl-1, serine-125 human interleukin-2)	Proleukin	Chiron Corp., Emeryville, CA
Alemtuzumab (IgG1κ anti CD52 antibody)	Campath	Millennium and ILEX Partners, LP, Cambridge, MA
Alitretinoin (9-cis-retinoic acid)	Panretin	Ligand Pharmaceuticals, Inc., San Diego CA
Allopurinol (1,5-dihydro-4 H -pyrazolo[3,4-d]pyrimidin-4-one monosodium salt)	Zyloprim	GlaxoSmithKline, Research Triangle Park, NC
Altretamine (N,N,N',N',N",N",- hexamethyl-1,3,5-triazine-2, 4, 6-triamine)	Hexalen	US Bioscience, West Conshohocken, PA
Amifostine (ethanethiol, 2-[(3-aminopropyl)amino]-, dihydrogen phosphate (ester))	Ethyol	US Bioscience
Anastrozole (1,3-Benzenediacetonitrile, a, a, a', a'-tetramethyl-5-(1H-1,2,4-triazol-1-ylmethyl))	Arimidex	AstraZeneca Pharmaceuticals, LP, Wilmington, DE
Arsenic trioxide	Trisenox	Cell Therapeutic, Inc., Seattle, WA
Asparaginase (L-asparagine amidohydrolase, type EC-2)	Elspar	Merck & Co., Inc., Whitehouse Station, NJ
BCG Live (lyophilized preparation of an attenuated strain of *Mycobacterium bovis* (*Bacillus Calmette-Gukin* [BCG], substrain Montreal)	TICE BCG	Organon Teknika, Corp., Durham, NC

bexarotene capsules (4-[1-(5,6,7,8-tetrahydro-3,5,5,8,8-pentamethyl-2-napthalenyl) ethenyl] benzoic acid)	Targretin	Ligand Pharmaceuticals
bexarotene gel	Targretin	Ligand Pharmaceuticals
Bleomycin (cytotoxic glycopeptide antibiotics produced by *Streptomyces verticillus*; bleomycin A_2 and bleomycin B_2)	Blenoxane	Bristol-Myers Squibb Co., NY, NY
Capecitabine (5'-deoxy-5-fluoro-N-[(pentyloxy)carbonyl]-cytidine)	Xeloda	Roche
Carboplatin (platinum, diammine [1,1-cyclobutanedicarboxylato(2-)-0, 0']-,(SP-4-2))	Paraplatin	Bristol-Myers Squibb
Carmustine (1,3-bis(2-chloroethyl)-1-nitrosourea)	BCNU, BiCNU	Bristol-Myers Squibb
Carmustine with Polifeprosan 20 Implant	Gliadel Wafer	Guilford Pharmaceuticals, Inc., Baltimore, MD
Celecoxib (as 4-[5-(4-methylphenyl)-3- (trifluoromethyl)-1H-pyrazol-1-yl] benzenesulfonamide)	Celebrex	Searle Pharmaceuticals, England
Chlorambucil (4-[bis(2chlorethyl)amino]benzenebutanoic acid)	Leukeran	GlaxoSmithKline
Cisplatin ($PtCl_2H_6N_2$)	Platinol	Bristol-Myers Squibb
Cladribine (2-chloro-2'-deoxy-b-D-adenosine)	Leustatin, 2-CdA	R.W. Johnson Pharmaceutical Research Institute, Raritan, NJ
Cyclophosphamide (2-[bis(2-chloroethyl)amino] tetrahydro-2H-13,2-oxazaphosphorine 2-oxide monohydrate)	Cytoxan, Neosar	Bristol-Myers Squibb
Cytarabine (1-b-D-Arabinofuranosylcytosine, $C_9H_{13}N_3O_5$)	Cytosar-U	Pharmacia & Upjohn Company
cytarabine liposomal	DepoCyt	Skye Pharmaceuticals, Inc., San Diego, CA
Dacarbazine (5-(3,3-dimethyl-l-triazeno)-imidazole-4-carboxamide (DTIC))	DTIC-Dome	Bayer AG, Leverkusen, Germany
Dactinomycin, actinomycin D (actinomycin produced by *Streptomyces parvullus*, $C_{62}H_{86}N_{12}O_{16}$)	Cosmegen	Merck
Darbepoetin alfa (recombinant peptide)	Aranesp	Amgen, Inc., Thousand Oaks, CA
daunorubicin liposomal ((8S-cis)-8-acetyl-10-[(3-amino-2,3,6-trideoxy-á-L-lyxo-hexopyranosyl)oxy]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-1-methoxy-5,12-naphthacenedione hydrochloride)	DanuoXome	Nexstar Pharmaceuticals, Inc., Boulder, CO

Daunorubicin HCl, daunomycin ((1 *S*,3 *S*)-3-Acetyl-1,2,3,4,6,11-hexahydro-3,5,12-trihydroxy-10-methoxy-6,11-dioxo-1-naphthacenyl 3-amino-2,3,6-trideoxy-(alpha)-L-*lyxo* -hexopyranoside hydrochloride)	Cerubidine	Wyeth Ayerst, Madison, NJ
Denileukin diftitox (recombinant peptide)	Ontak	Seragen, Inc., Hopkinton, MA
Dexrazoxane ((S)-4,4'-(1-methyl-1,2-ethanediyl)bis-2,6-piperazinedione)	Zinecard	Pharmacia & Upjohn Company
Docetaxel ((2R,3S)-N-carboxy-3-phenylisoserine, N-tert-butyl ester, 13-ester with 5b-20-epoxy-12a,4,7b,10b,13a-hexahydroxytax- 11-en-9-one 4-acetate 2-benzoate, trihydrate)	Taxotere	Aventis Pharmaceuticals, Inc., Bridgewater, NJ
Doxorubicin HCl (8S,10S)-10-[(3-amino-2,3,6-trideoxy-a-L-lyxo-hexopyranosyl)oxy] -8-glycolyl-7,8,9,10-tetrahydro-6,8,11- trihydroxy-1-methoxy-5,12-naphthacenedione hydrochloride)	Adriamycin, Rubex	Pharmacia & Upjohn Company
doxorubicin	Adriamycin PFS Intravenous injection	Pharmacia & Upjohn Company
doxorubicin liposomal	Doxil	Sequus Pharmaceuticals, Inc., Menlo park, CA
dromostanolone propionate (17b-Hydroxy-2a-methyl-5a-androstan-3-one propionate)	Dromostanolone	Eli Lilly & Company, Indianapolis, IN
dromostanolone propionate	Masterone injection	Syntex, Corp., Palo Alto, CA
Elliott's B Solution	Elliott's B Solution	Orphan Medical, Inc
Epirubicin ((8S-cis)-10-[(3-amino-2,3,6-trideoxy-a-L-arabino-hexopyranosyl)oxy]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-8- (hydroxyacetyl)-1-methoxy-5,12-naphthacenedione hydrochloride)	Ellence	Pharmacia & Upjohn Company
Epoetin alfa (recombinant peptide)	Epogen	Amgen, Inc
Estramustine (estra-1,3,5(10)-triene-3,17-diol(17(beta))-, 3-[bis(2-chloroethyl)carbamate] 17-(dihydrogen phosphate), disodium salt, monohydrate, or. estradiol 3-[bis(2-chloroethyl)carbamate] 17-(dihydrogen phosphate), disodium salt, monohydrate)	Emcyt	Pharmacia & Upjohn Company
Etoposide phosphate (4'-Demethylepipodophyllotoxin 9-[4,6-O-(R)-ethylidene-(beta)-D-glucopyranoside], 4'-(dihydrogen phosphate))	Etopophos	Bristol-Myers Squibb
etoposide, VP-16 (4'-demethylepipodophyllotoxin 9-[4,6-0-(R)-ethylidene-(beta)-D-glucopyranoside])	Vepesid	Bristol-Myers Squibb

Exemestane (6-methylenandrosta-1,4-diene-3, 17-dione)	Aromasin	Pharmacia & Upjohn Company
Filgrastim (r-metHuG-CSF)	Neupogen	Amgen, Inc
floxuridine (intraarterial) (2'-deoxy-5-fluorouridine)	FUDR	Roche
Fludarabine (fluorinated nucleotide analog of the antiviral agent vidarabine, 9-b -D-arabinofuranosyladenine (ara-A))	Fludara	Berlex Laboratories, Inc., Cedar Knolls, NJ
Fluorouracil, 5-FU (5-fluoro-2,4(1H,3H)-pyrimidinedione)	Adrucil	ICN Pharmaceuticals, Inc., Humacao, Puerto Rico
Fulvestrant (7-alpha-[9-(4,4,5,5,5-penta fluoropentylsulphinyl) nonyl]estra-1,3,5-(10)- triene-3,17-beta-diol)	Faslodex	IPR Pharmaceuticals, Guayama, Puerto Rico
Gemcitabine (2'-deoxy-2', 2'-difluorocytidine monohydrochloride (b-isomer))	Gemzar	Eli Lilly
Gemtuzumab Ozogamicin (anti-CD33 hP67.6)	Mylotarg	Wyeth Ayerst
Goserelin acetate (acetate salt of [D-Ser(But)^6,Azgly^{10}]LHRH; pyro-Glu-His-Trp-Ser-Tyr-D-Ser(But)-Leu-Arg-Pro-Azgly-NH2 acetate [$C_{59}H_{84}N_{18}O_{14} \cdot (C_2H_4O_2)_x$	Zoladex Implant	AstraZeneca Pharmaceuticals
Hydroxyurea	Hydrea	Bristol-Myers Squibb
Ibritumomab Tiuxetan (immunoconjugate resulting from a thiourea covalent bond between the monoclonal antibody Ibritumomab and the linker-chelator tiuxetan [N-[2-bis(carboxymethyl)amino]-3-(p-isothiocyanatophenyl)- propyl]-[N-[2-bis(carboxymethyl)amino]-2-(methyl) - ethyl]glycine)	Zevalin	Biogen IDEC, Inc., Cambridge MA
Idarubicin (5, 12-Naphthacenedione, 9-acetyl-7-[(3-amino-2,3,6-trideoxy-(alpha)-L- lyxo - hexopyranosyl)oxy]-7,8,9,10-tetrahydro-6,9,11-trihydroxyhydrochloride, (7S- cis))	Idamycin	Pharmacia & Upjohn Company
Ifosfamide (3-(2-chloroethyl)-2-[(2-chloroethyl)amino]tetrahydro-2H-1,3,2-oxazaphosphorine 2-oxide)	IFEX	Bristol-Myers Squibb
Imatinib Mesilate (4-[(4-Methyl-1-piperazinyl)methyl]-N-[4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-phenyl]benzamide methanesulfonate)	Gleevec	Novartis AG, Basel, Switzerland
Interferon alfa-2a (recombinant peptide)	Roferon-A	Hoffmann-La Roche, Inc., Nutley, NJ
Interferon alfa-2b (recombinant peptide)	Intron A (Lyophilized Betaseron)	Schering AG, Berlin, Germany
Irinotecan HCl ((4S)-4,11-diethyl-4-hydroxy-9-[(4- piperi-	Camptosar	Pharmacia & Upjohn Company

dinopiperidino)carbonyloxy]-1H-pyrano[3', 4': 6,7] indolizino[1,2-b] quinoline-3,14(4H, 12H) dione hydrochloride trihydrate)		
Letrozole (4,4'-(1H-1,2,4 -Triazol-1-ylmethylene) dibenzonitrile)	Femara	Novartis
Leucovorin (L-Glutamic acid, N[4[[(2amino-5-formyl-1,4,5,6,7,8 hexahydro4oxo6-pteridinyl)methyl]amino]benzoyl], calcium salt (1:1))	Wellcovorin, Leucovorin	Immunex, Corp., Seattle, WA
Levamisole HCl ((-)-(S)-2,3,5, 6-tetrahydro-6-phenylimidazo [2,1-b] thiazole monohydrochloride $C_{11}H_{12}N_2S \cdot HCl$)	Ergamisol	Janssen Research Foundation, Titusville, NJ
Lomustine (1-(2-chloro-ethyl)-3-cyclohexyl-1-nitrosourea)	CeeNU	Bristol-Myers Squibb
Meclorethamine, nitrogen mustard (2-chloro-N-(2-chloroethyl)-N-methylethanamine hydrochloride)	Mustargen	Merck
Megestrol acetate 17α(acetyloxy)- 6- methylpregna- 4,6- diene-3,20- dione	Megace	Bristol-Myers Squibb
Melphalan, L-PAM (4-[bis(2-chloroethyl) amino]-L-phenylalanine)	Alkeran	GlaxoSmithKline
Mercaptopurine, 6-MP (1,7-dihydro-6 H -purine-6-thione monohydrate)	Purinethol	GlaxoSmithKline
Mesna (sodium 2-mercaptoethane sulfonate)	Mesnex	Asta Medica
Methotrexate (N-[4-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-L-glutamic acid)	Methotrexate	Lederle Laboratories
Methoxsalen (9-methoxy-7H-furo[3,2-g][1]-benzopyran-7-one)	Uvadex	Therakos, Inc., Way Exton, Pa
Mitomycin C	Mutamycin	Bristol-Myers Squibb
mitomycin C	Mitozytrex	SuperGen, Inc., Dublin, CA
Mitotane (1,1-dichloro-2-(o-chlorophenyl)-2-(p-chlorophenyl) ethane)	Lysodren	Bristol-Myers Squibb
Mitoxantrone (1,4-dihydroxy-5,8-bis[[2- [(2-hydroxyethyl)amino]ethyl]amino]-9,10-anthracenedione dihydrochloride)	Novantrone	Immunex Corporation
Nandrolone phenpropionate	Durabolin-50	Organon, Inc., West Orange, NJ
Nofetumomab	Verluma	Boehringer Ingelheim Pharma KG, Germany
Oprelvekin (IL-11)	Neumega	Genetics Institute, Inc., Alexandria, VA
Oxaliplatin (cis-[(1R,2R)-1,2-cyclohexanediamine-N,N'] [oxalato(2-)-O,O'] platinum)	Eloxatin	Sanofi Synthelabo, Inc., NY, NY

Paclitaxel (5ß, 20-Epoxy-1,2a, 4,7ß, 10ß, 13a-hexahydroxytax-11-en-9-one 4,10-diacetate 2-benzoate 13-ester with (2R, 3 S)- N-benzoyl-3-phenylisoserine)	TAXOL	Bristol-Myers Squibb
Pamidronate (phosphonic acid (3-amino-1-hydroxypropylidene) bis-, disodium salt, pentahydrate, (APD))	Aredia	Novartis
Pegademase ((monomethoxypolyethylene glycol succinimidyl) 11 - 17 -adenosine deaminase)	Adagen (Pegademase Bovine)	Enzon Pharmaceuticals, Inc., Bridgewater, NJ
Pegaspargase (monomethoxypolyethylene glycol succinimidyl L-asparaginase)	Oncaspar	Enzon
Pegfilgrastim (covalent conjugate of recombinant methionyl human G-CSF (Filgrastim) and monomethoxypolyethylene glycol)	Neulasta	Amgen, Inc
Pentostatin	Nipent	Parke-Davis Pharmaceutical Co., Rockville, MD
Pipobroman	Vercyte	Abbott Laboratories, Abbott Park, IL
Plicamycin, Mithramycin (antibiotic produced by *Streptomyces plicatus*)	Mithracin	Pfizer, Inc., NY, NY
Porfimer sodium	Photofrin	QLT Phototherapeutics, Inc., Vancouver, Canada
Procarbazine (N-isopropyl-µ-(2-methylhydrazino)-p-toluamide monohydrochloride)	Matulane	Sigma Tau Pharmaceuticals, Inc., Gaithersburg, MD
Quinacrine (6-chloro-9-(1 –methyl-4-diethyl-amine) butylamino-2-methoxyacridine)	Atabrine	Abbott Labs
Rasburicase (recombinant peptide)	Elitek	Sanofi-Synthelabo, Inc.,
Rituximab (recombinant anti-CD20 antibody)	Rituxan	Genentech, Inc., South San Francisco, CA
Sargramostim (recombinant peptide)	Prokine	Immunex Corp
Streptozocin (streptozocin 2 –deoxy - 2 - [[(methylnitrosoamino)carbonyl]amino] - a(and b) - D - glucopyranose and 220 mg citric acid anhydrous)	Zanosar	Pharmacia & Upjohn Company
Talc ($Mg_3Si_4O_{10}$ $(OH)_2$)	Sclerosol	Bryan, Corp., Woburn, MA
Tamoxifen ((Z)2-[4-(1,2-diphenyl-1-butenyl) phenoxy]-N, N-dimethylethanamine 2-hydroxy-1,2,3-propanetricarboxylate (1:1))	Nolvadex	AstraZeneca Pharmaceuticals

Temozolomide (3,4-dihydro-3-methyl-4-oxoimidazo[5,1-d]-as-tetrazine-8-carboxamide)	Temodar	Schering
teniposide, VM-26 (4'-demethylepipodophyllotoxin 9-[4,6-0-(R)-2-thenylidene-(beta)-D-glucopyranoside])	Vumon	Bristol-Myers Squibb
Testolactone (13-hydroxy-3-oxo-13,17-secoandrosta-1,4-dien-17-oic acid [dgr]-lactone)	Teslac	Bristol-Myers Squibb
Thioguanine, 6-TG (2-amino-1,7-dihydro-6 H - purine-6-thione)	Thioguanine	GlaxoSmithKline
Thiotepa (Aziridine, 1,1',1"-phosphinothioylidynetris-, or Tris (1-aziridinyl) phosphine sulfide)	Thioplex	Immunex Corporation
Topotecan HCl ((S)-10-[(dimethylamino) methyl]-4-ethyl-4,9-dihydroxy-1H-pyrano[3', 4': 6,7] indolizino [1,2-b] quinoline-3,14-(4H,12H)-dione monohydrochloride)	Hycamtin	GlaxoSmithKline
Toremifene (2-(p-[(Z)-4-chloro-1,2-diphenyl-1-butenyl]-phenoxy)-N,N-dimethylethylamine citrate (1:1))	Fareston	Roberts Pharmaceutical Corp., Eatontown, NJ
Tositumomab, I 131 Tositumomab (recombinant murine immunotherapeutic monoclonal IgG_{2a} lambda anti-CD20 antibody (I 131 is a radioimmunotherapeutic antibody))	Bexxar	Corixa Corp., Seattle, WA
Trastuzumab (recombinant monoclonal IgG_1 kappa anti-HER2 antibody)	Herceptin	Genentech, Inc
Tretinoin, ATRA (all-trans retinoic acid)	Vesanoid	Roche
Uracil Mustard	Uracil Mustard Capsules	Roberts Labs
Valrubicin, N-trifluoroacetyladriamycin-14-valerate ((2S-cis)-2- [1,2,3,4,6,11-hexahydro-2,5,12-trihydroxy-7 methoxy-6,11-dioxo-[[4 2,3,6-trideoxy-3- [(trifluoroacetyl)-amino-α-L-*lyxo*-hexopyranosyl]oxyl]-2-naphthacenyl]-2-oxoethyl pentanoate)	Valstar	Anthra --> Medeva
Vinblastine, Leurocristine ($C_{46}H_{56}N_4O_{10}•H_2SO_4$)	Velban	Eli Lilly
Vincristine ($C_{46}H_{56}N_4O_{10}•H_2SO_4$)	Oncovin	Eli Lilly
Vinorelbine (3',4'-didehydro-4'-deoxy-C'-norvincaleukoblastine [R-(R*,R*)-2,3-dihydroxybutanedioate (1:2)(salt)])	Navelbine	GlaxoSmithKline
Zoledronate, Zoledronic acid ((1-Hydroxy-2-imidazol-1-yl-phosphonoethyl) phosphonic acid monohydrate)	Zometa	Novartis

[0056] Preferred conventional anticancer agents for use in administration with the present compounds include, but are not limited to, adriamycin, 5-fluorouracil, etoposide, camptothecin, actinomycin D, mitomycin C, cisplatin, docetaxel, gemcitabine, carboplatin, oxaliplatin, bortezomib, gefitinib, and bevacizumab. These agents can be prepared and used singularly, in combined therapeutic compositions, in kits, or in combination with immunotherapeutic agents, and the like.

[0057] For a more detailed description of anticancer agents and other therapeutic agents, those skilled in the art are referred to any number of instructive manuals including, but not limited to, the Physician's Desk Reference and to Goodman and Gilman's "Pharmaceutical Basis of Therapeutics" ninth edition, Eds. Hardman *et al.*, 1996.

[0058] The present invention provides methods for administering (-)-gossypol co-crystal with radiation therapy. The invention is not limited by the types, amounts, or delivery and administration systems used to deliver the therapeutic dose of radiation to an animal. For example, the animal may receive photon radiotherapy, particle beam radiation therapy, radioisotope therapy (*e.g.*, radioconjugates with monoclonal antibodies), other types of radiotherapies, and combinations thereof. In some embodiments, the radiation is delivered to the animal using a linear accelerator. In still other embodiments, the radiation is delivered using a gamma knife.

[0059] The source of radiation can be external or internal to the animal. External radiation therapy is most common and involves directing a beam of high-energy radiation to a tumor site through the skin using, for instance, a linear accelerator. While the beam of radiation is localized to the tumor site, it is nearly impossible to avoid exposure of normal, healthy tissue. However, external radiation is usually well tolerated by patients. Internal radiation therapy involves implanting a radiation-emitting source, such as beads, wires, pellets, capsules, particles, and the like, inside the body at or near the tumor site including the use of delivery systems that specifically target cancer cells

(*e.g.*, using particles attached to cancer cell binding ligands). Such implants can be removed following treatment, or left in the body inactive. Types of internal radiation therapy include, but are not limited to, brachytherapy, interstitial irradiation, intracavity irradiation, radioimmunotherapy, and the like.

[0060] The animal may optionally receive radiosensitizers (*e.g.*, metronidazole, misonidazole, intra-arterial Budr, intravenous iododeoxyuridine (IudR), nitroimidazole, 5-substituted-4-nitroimidazoles, 2H-isoindolediones, [[(2-bromoethyl)-amino]methyl]-nitro-1H-imidazole-1-ethanol, nitroaniline derivatives, DNA-affinic hypoxia selective cytotoxins, halogenated DNA ligand, 1,2,4 benzotriazine oxides, 2-nitroimidazole derivatives, fluorine-containing nitroazole derivatives, benzamide, nicotinamide, acridine-intercalator, 5-thiotretrazole derivative, 3-nitro-1,2,4-triazole, 4,5-dinitroimidazole derivative, hydroxylated texaphrins, cisplatin, mitomycin, tiripazamine, nitrosourea, mercaptopurine, methotrexate, fluorouracil, bleomycin, vincristine, carboplatin, epirubicin, doxorubicin, cyclophosphamide, vindesine, etoposide, paclitaxel, heat (hyperthermia), and the like), radioprotectors (*e.g.*, cysteamine, aminoalkyl dihydrogen phosphorothioates, amifostine (WR 2721), IL-1, IL-6, and the like). Radiosensitizers enhance the killing of tumor cells. Radioprotectors protect healthy tissue from the harmful effects of radiation.

[0061] Any type of radiation can be administered to a patient, so long as the dose of radiation is tolerated by the patient without unacceptable negative side-effects. Suitable types of radiotherapy include, for example, ionizing (electromagnetic) radiotherapy (*e.g.*, X-rays or gamma rays) or particle beam radiation therapy (*e.g.*, high linear energy radiation). Ionizing radiation is defined as radiation comprising particles or photons that have sufficient energy to produce ionization, *i.e.*, gain or loss of electrons (as described in, for example, U.S. 5,770,581 incorporated herein by reference in its entirety). The effects of radiation can be at least partially controlled by the clinician. The

dose of radiation is preferably fractionated for maximal target cell exposure and reduced toxicity.

[0062] The total dose of radiation administered to an animal preferably is about .01 Gray (Gy) to about 100 Gy. More preferably, about 10 Gy to about 65 Gy (*e.g.*, about 15 Gy, 20 Gy, 25 Gy, 30 Gy, 35 Gy, 40 Gy, 45 Gy, 50 Gy, 55 Gy, or 60 Gy) are administered over the course of treatment. While in some embodiments a complete dose of radiation can be administered over the course of one day, the total dose is ideally fractionated and administered over several days. Desirably, radiotherapy is administered over the course of at least about 3 days, *e.g.*, at least 5, 7, 10, 14, 17, 21, 25, 28, 32, 35, 38, 42, 46, 52, or 56 days (about 1-8 weeks). Accordingly, a daily dose of radiation will comprise approximately 1-5 Gy (*e.g.*, about 1 Gy, 1.5 Gy, 1.8 Gy, 2 Gy, 2.5 Gy, 2.8 Gy, 3 Gy, 3.2 Gy, 3.5 Gy, 3.8 Gy, 4 Gy, 4.2 Gy, or 4.5 Gy), preferably 1-2 Gy (*e.g.*, 1.5-2 Gy). The daily dose of radiation should be sufficient to induce destruction of the targeted cells. If stretched over a period, radiation preferably is not administered every day, thereby allowing the animal to rest and the effects of the therapy to be realized. For example, radiation desirably is administered on 5 consecutive days, and not administered on 2 days, for each week of treatment, thereby allowing 2 days of rest per week. However, radiation can be administered 1 day/week, 2 days/week, 3 days/week, 4 days/week, 5 days/week, 6 days/week, or all 7 days/week, depending on the animal's responsiveness and any potential side effects. Radiation therapy can be initiated at any time in the therapeutic period. Preferably, radiation is initiated in week 1 or week 2, and is administered for the remaining duration of the therapeutic period. For example, radiation is administered in weeks 1-6 or in weeks 2-6 of a therapeutic period comprising 6 weeks for treating, for instance, a solid tumor. Alternatively, radiation is administered in weeks 1-5 or weeks 2-5 of a therapeutic period comprising 5 weeks. These exemplary radiotherapy administration schedules are not intended, however, to limit the present invention.

[0063] Antimicrobial therapeutic agents may also be used as therapeutic agents in the present invention. Any agent that can kill, inhibit, or otherwise attenuate the function of microbial organisms may be used, as well as any agent contemplated to have such activities. Antimicrobial agents include, but are not limited to, natural and synthetic antibiotics, antibodies, inhibitory proteins (*e.g.*, defensins), antisense nucleic acids, membrane disruptive agents and the like, used alone or in combination. Indeed, any type of antibiotic may be used including, but not limited to, antibacterial agents, antiviral agents, antifungal agents, and the like.

[0064] In some embodiments of the present invention, (-)-gossypol co-crystal and one or more therapeutic agents or anticancer agents are administered to an animal under one or more of the following conditions: at different periodicities, at different durations, at different concentrations, by different administration routes, *etc.* In some embodiments, (-)-gossypol co-crystal is administered prior to the therapeutic or anticancer agent, *e.g.*, 0.5, 1, 2 3, 4, 5, 10, 12, or 18 hours, 1, 2, 3, 4, 5, or 6 days, 1, 2, 3, or 4 weeks prior to the administration of the therapeutic or anticancer agent. In some embodiments, (-)-gossypol co-crystal is administered after the therapeutic or anticancer agent, *e.g.*, 0.5, 1, 2 3, 4, 5, 10, 12, or 18 hours, 1, 2, 3, 4, 5, or 6 days, 1, 2, 3, or 4 weeks after the administration of the anticancer agent. In some embodiments, (-)-gossypol co-crystal and the therapeutic or anticancer agent are administered concurrently but on different schedules, *e.g.*, (-)-gossypol co-crystal is administered daily while the therapeutic or anticancer agent is administered once a week, once every two weeks, once every three weeks, or once every four weeks. In other embodiments, (-)-gossypol co-crystal is administered once a week while the therapeutic or anticancer agent is administered daily, once a week, once every two weeks, once every three weeks, or once every four weeks.

[0065] Pharmaceutical compositions can be produced by combining (-)-gossypol co-crystal in a therapeutically effective amount to induce apoptosis in cells or to sensitize cells to inducers of apoptosis with a pharmaceutically

acceptable carrier. The novel pharmaceutical compositions of the present invention comprise intact (-)-gossypol co-crystal. In some embodiments, the pharmaceutical compositions comprise (-)-gossypol co-crystal in combination with a liquid in which the co-crystal is substantially insoluble (*e.g.*, water) such that a suspension is formed.

[0066] Compositions within the scope of this invention include all compositions wherein the compositions of the present invention are contained in an amount which is effective to achieve its intended purpose. While individual needs vary, determination of optimal ranges of effective amounts of each component is within the skill of the art. Typically, the compositions may be administered to mammals, *e.g.* humans, orally at a dose of 0.0025 to 50 mg/kg, or an equivalent amount of the pharmaceutically acceptable salt thereof, per day of the body weight of the mammal being treated for disorders responsive to induction of apoptosis. Preferably, about 0.01 to about 10 mg/kg is orally administered to treat, ameliorate, or prevent such disorders. For intramuscular injection, the dose is generally about one-half of the oral dose. For example, a suitable intramuscular dose would be about 0.0025 to about 25 mg/kg, and most preferably, from about 0.01 to about 5 mg/kg.

[0067] The unit oral dose may comprise from about 0.01 to about 200 mg, preferably about 0.1 to about 100 mg of the composition. The unit dose may be administered one or more times daily as one or more tablets or capsules each containing from about 0.1 to about 100 mg, conveniently about 0.25 to 50 mg of the composition.

[0068] In a topical formulation, the composition may be present at a concentration of about 0.01 to 100 mg per gram of carrier. In a preferred embodiment, the composition is present at a concentration of about 0.07-1.0 mg/ml, more preferably, about 0.1-0.5 mg/ml, most preferably, about 0.4 mg/ml.

[0069] In addition to administering (-)-gossypol co-crystal as a raw chemical, the compositions of the invention may be administered as part of a pharmaceutical preparation containing suitable pharmaceutically acceptable

carriers comprising excipients and auxiliaries which facilitate processing of the compositions into preparations which can be used pharmaceutically. Preferably, the preparations, particularly those preparations which can be administered orally or topically and which can be used for the preferred type of administration, such as tablets, dragees, slow release lozenges and capsules, mouth rinses and mouth washes, gels, liquid suspensions, hair rinses, hair gels, shampoos and also preparations which can be administered rectally, such as suppositories, as well as suitable solutions for administration by injection, topically or orally, contain from about 0.01 to 99 percent, preferably from about 0.25 to 75 percent of active compound(s), together with the excipient.

[0070] The pharmaceutical compositions of the invention may be administered to any animal which may experience the beneficial effects of the compounds of the invention. Foremost among such animals are mammals, *e.g.*, humans, although the invention is not intended to be so limited. Other animals include veterinary animals (cows, sheep, pigs, horses, dogs, cats and the like).

[0071] The compositions and pharmaceutical compositions thereof may be administered by any means that achieve their intended purpose. For example, administration may be by parenteral, subcutaneous, intravenous, intramuscular, intraperitoneal, transdermal, buccal, intrathecal, intracranial, intranasal, or topical routes. Alternatively, or concurrently, administration may be by the oral route. The dosage administered will be dependent upon the age, health, and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment, and the nature of the effect desired.

[0072] The pharmaceutical preparations of the present invention are manufactured in a manner which is itself known, for example, by means of conventional mixing, granulating, dragee-making, dissolving, or lyophilizing processes. Thus, pharmaceutical preparations for oral use can be obtained by combining the active compounds with solid excipients, optionally grinding the resulting mixture and processing the mixture of granules, after adding suitable auxiliaries, if desired or necessary, to obtain tablets or dragee cores.

[0073] Suitable excipients are, in particular, fillers such as saccharides, for example lactose or sucrose, mannitol or sorbitol, cellulose preparations and/or calcium phosphates, for example tricalcium phosphate or calcium hydrogen phosphate, as well as binders such as starch paste, using, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, tragacanth, methyl cellulose, hydroxypropylmethylcellulose, sodium carboxymethylcellulose, and/or polyvinyl pyrrolidone. If desired, disintegrating agents may be added such as the above-mentioned starches and also carboxymethyl-starch, cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof, such as sodium alginate. Auxiliaries are, above all, flow-regulating agents and lubricants, for example, silica, talc, stearic acid or salts thereof, such as magnesium stearate or calcium stearate, and/or polyethylene glycol. Dragee cores are provided with suitable coatings which, if desired, are resistant to gastric juices. For this purpose, concentrated saccharide solutions may be used, which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, polyethylene glycol and/or titanium dioxide, lacquer solutions and suitable organic solvents or solvent mixtures. In order to produce coatings resistant to gastric juices, solutions of suitable cellulose preparations such as acetylcellulose phthalate or hydroxypropylmethyl-cellulose phthalate, are used. Dye stuffs or pigments may be added to the tablets or dragee coatings, for example, for identification or in order to characterize combinations of active compound doses.

[0074] Other pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer such as glycerol or sorbitol. The push-fit capsules can contain the active compounds in the form of granules which may be mixed with fillers such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds are preferably dissolved or suspended in suitable liquids, such as fatty oils, or liquid paraffin. In addition, stabilizers may be added.

[0075] Possible pharmaceutical preparations which can be used rectally include, for example, suppositories, which consist of a combination of one or more of the active compounds with a suppository base. Suitable suppository bases are, for example, natural or synthetic triglycerides, or paraffin hydrocarbons. In addition, it is also possible to use gelatin rectal capsules which consist of a combination of the active compounds with a base. Possible base materials include, for example, liquid triglycerides, polyethylene glycols, or paraffin hydrocarbons.

[0076] Suitable formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form, for example, water-soluble salts and alkaline solutions. In addition, suspensions of the active compounds as appropriate oily injection suspensions may be administered. Suitable lipophilic solvents or vehicles include fatty oils, for example, sesame oil, or synthetic fatty acid esters, for example, ethyl oleate or triglycerides or polyethylene glycol-400. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension include, for example, sodium carboxymethyl cellulose, sorbitol, and/or dextran. Optionally, the suspension may also contain stabilizers.

[0077] The topical compositions of this invention are formulated preferably as oils, creams, lotions, ointments and the like by choice of appropriate carriers. Suitable carriers include vegetable or mineral oils, white petrolatum (white soft paraffin), branched chain fats or oils, animal fats and high molecular weight alcohol (greater than C_{12}). The preferred carriers are those in which the active ingredient is soluble. Emulsifiers, stabilizers, humectants and antioxidants may also be included as well as agents imparting color or fragrance, if desired. Additionally, transdermal penetration enhancers can be employed in these topical formulations. Examples of such enhancers can be found in U.S. Pat. Nos. 3,989,816 and 4,444,762.

[0078] Creams are preferably formulated from a mixture of mineral oil, self-emulsifying beeswax and water in which mixture the active ingredient, dissolved in a small amount of an oil such as almond oil, is admixed. A

typical example of such a cream is one which includes about 40 parts water, about 20 parts beeswax, about 40 parts mineral oil and about 1 part almond oil.

[0079] Ointments may be formulated by mixing a suspension of the active ingredient in a vegetable oil such as almond oil with warm soft paraffin and allowing the mixture to cool. A typical example of such an ointment is one which includes about 30% almond oil and about 70% white soft paraffin by weight.

[0080] Lotions may be conveniently prepared by preparing a suspension of the active ingredient in a suitable high molecular weight alcohol such as propylene glycol or polyethylene glycol.

[0081] The following examples are illustrative, but not limiting, of the method and compositions of the present invention. Other suitable modifications and adaptations of the variety of conditions and parameters normally encountered in clinical therapy and which are obvious to those skilled in the art are within the spirit and scope of the invention.

EXAMPLE 1

Preparation of (-)-Gossypol Acetic Acid Co-crystal

[0082] All chemicals and reagents were purchased from Aldrich Chemical Co. or Lancaster Synthesis Inc. and used without further purification. (-)-Gossypol (1 g) was dissolved in acetone (6 ml) and filtered. Acetic acid was added into the constantly stirred filtrate until the solution turned turbid. The mixture was left at room temperature for 2 hours and then at 4°C for 2 hours. The co-crystals were collected by filtration using a Buchner funnel under reduced pressure and washed with a small amount of hexane. Pure (-)-gossypol acetic acid was first dried in a lightproof container and further dried in a vacuum drier at 40°C for 24 hours.

EXAMPLE 2

Characterization of (-)-Gossypol Acetic Acid Co-crystals

[0083] (-)-Gossypol acetic acid co-crystals were yellow or pale yellow and needle shaped. The co-crystals were readily soluble in acetone and ether, slightly soluble in chloroform and ethanol, and sparsely soluble in petroleum. The co-crystals were insoluble in water. The uncorrected melting point of the co-crystals was determined to be 178-180°C using a Mel-Temp apparatus.

[0084] ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra of the co-crystals (Figs. 1 and 2) were recorded on a Bruker 300 instrument. Samples were dissolved in an appropriate deuterated solvent ($CDCl_3$). Proton chemical shifts were reported as parts per million (δ) relative to tetramethylsilane (0.00 ppm), which was used as an internal standard. Chemical shifts for ^{13}C NMR spectra were reported as δ relative to deuterated chloroform ($CDCl_3$, 77.0 ppm). ^{1}H NMR (300 MHz, $CDCl_3$) δ 15.21 (s, 2H), 11.16 (s, 2H), 7.80 (s, 2H), 6.45 (s, 2H), 5.79 (s, 2H), 4.08-3.80 (m, 2H), 2.18 (s, 6H), 2.11 (s, 3H), 1.58 (d, J=6.8 Hz, 12H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 199.4, 176.8, 156.0, 150.5, 143.4, 134.1, 133.7, 129.7, 118.1, 115.9, 114.6, 111.8, 27.9, 20.7, 20.3, 20.2. Based on the ^{1}H NMR spectrum, the co-crystal was determined to be a complex of (-)-gossypol with acetic acid at a molar ratio of 1:1.

[0085] The infrared spectrum (Fig. 3) of the co-crystals was recorded on a Perkin-Elmer FT-IR spectrometer. IR(KBr) 3421, 2959, 2929, 1710, 1611, 1577, 1440, 1379, 1339, 1269, 1176, 1052, 841, 772 cm^{-1}.

[0086] The electrospray mass spectrum (Fig. 4) of the co-crystals was performed on a Micromass AutoSpec Ultima Magnetic sector mass spectrometer. MS m/z 541 $(M+Na)^{+}$.

[0087] The X-ray powder diffraction spectrum (Fig. 5) of the co-crystals was recorded on a Scintag X-ray powder diffractometer. Based on the spectrum, the co-crystal was determined to be a complex of (-)-gossypol with acetic acid at a molar ratio of 1:1.

[0088] Having now fully described the invention, it will be understood by those of skill in the art that the same can be performed within a wide and equivalent range of conditions, formulations, and other parameters without affecting the scope of the invention or any embodiment thereof. All patents, patent applications and publications cited herein are fully incorporated by reference herein in their entirety.

WHAT IS CLAIMED IS:

1. A composition comprising co-crystals of (-)-gossypol with a C_{1-8} carboxylic acid or C_{1-8} sulfonic acid.

2. The composition of claim 1, wherein said C_{1-8} carboxylic acid is selected from the group consisting of formic acid, acetic acid, propionic acid, n-butyric acid, t-butyric acid, n-pentanoic acid, 2-pentanoic acid, n-hexanoic acid, 2-hexanoic acid, n-heptanoic acid, n-octanoic acid, acrylic acid, succinic acid, fumaric acid, malic acid, tartaric acid, citric acid, lactic acid, and benzoic acid.

3. The composition of claim 2, wherein said C_{1-8} carboxylic acid or C_{1-8} sulfonic acid is acetic acid.

4. The composition of claim 1, wherein said C_{1-8} sulfonic acid is selected from the group consisting of methanesulfonic acid, ethanesulfonic acid, n-propanesulfonic acid, 2-propanesulfonic acid, n-butanesulfonic acid, n-pentanesulfonic acid n-hexanesulfonic acid, n-heptanesulfonic acid, n-octanesulfonic acid, and benzenesulfonic acid.

5. The composition of claim 1, wherein said (-)-gossypol and said C_{1-8} carboxylic acid or C_{1-8} sulfonic acid are present in the composition at a molar ratio in the range of about 10:1 to about 1:10.

6. The composition of claim 5, wherein said (-)-gossypol and said C_{1-8} carboxylic acid or C_{1-8} sulfonic acid are present in the composition at a molar ratio of about 1:1.

7. The composition of claim 6, comprising (-)-gossypol and acetic acid in a 1:1 molar ratio.

8. The composition of claim 7, which is characterized by integration of ^{1}H NMR spectrum at δ 2.11 (s, 3H) which is one methyl signal of acetic acid and δ 2.18 (s, 6H) which is two methyl signals of gossypol.

9. A method of preparing a composition comprising co-crystals of (-)-gossypol with a C_{1-8} carboxylic acid or C_{1-8} sulfonic acid, said method comprising dissolving (-)-gossypol in acetone to form a solution, filtering the solution, adding a C_{1-8} carboxylic acid or C_{1-8} sulfonic acid into the solution with mixing until the solution turns turbid, leaving the turbid solution at room temperature then at a reduced temperature to form co-crystals, collecting the co-crystals, washing the co-crystals with a solvent, and drying the co-crystals.

10. The method of claim 9, wherein said C_{1-8} carboxylic acid or C_{1-8} sulfonic acid is acetic acid.

11. A pharmaceutical composition comprising the composition of claim 1 and a pharmaceutically acceptable carrier.

12. A method of preparing a pharmaceutical composition comprising combining the composition of claim 1 with a pharmaceutically acceptable carrier.

13. A method of treating a hyperproliferative disease or cancer in an animal, comprising administering to said animal a therapeutically effective amount of the composition of claim 1.

14. The method of claim 13, further comprising administering to said animal an inducer of apoptosis.

15. The method of claim 13, wherein said inducer of apoptosis is a chemotherapeutic agent.

16. The method of claim 13, wherein said inducer of apoptosis is radiation.

17. The method of claim 13, wherein said composition is administered prior to said inducer of apoptosis.

18. The method of claim 13, wherein said composition is administered concurrently with said inducer of apoptosis.

19. The method of claim 13, wherein said composition is administered after said inducer of apoptosis.

20. A method of treating a viral, microbial, or parasitic infection in an animal, comprising administering to said animal a therapeutically effective amount of the composition of claim 1.

21. A method of treating, ameliorating, or preventing a disorder responsive to the induction of apoptosis in an animal, comprising administering to said animal a therapeutically effective amount of the composition of claim 1.

22. The method of claim 21, further comprising administering to said animal an inducer of apoptosis.

23. A kit comprising a composition of claim 1 and instructions for administering said composition to an animal.

24. The kit of claim 23, wherein said composition is in the form of a pharmaceutical composition comprising a pharmaceutically acceptable carrier.

25. The kit of claim 23, further comprising an inducer of apoptosis.

26. The kit of claim 25, wherein said inducer of apoptosis is a chemotherapeutic agent.

27. The kit of claim 23, wherein said instructions are for administering said composition to an animal having a hyperproliferative disease.

28. The kit of claim 27, wherein said hyperproliferative disease is cancer.

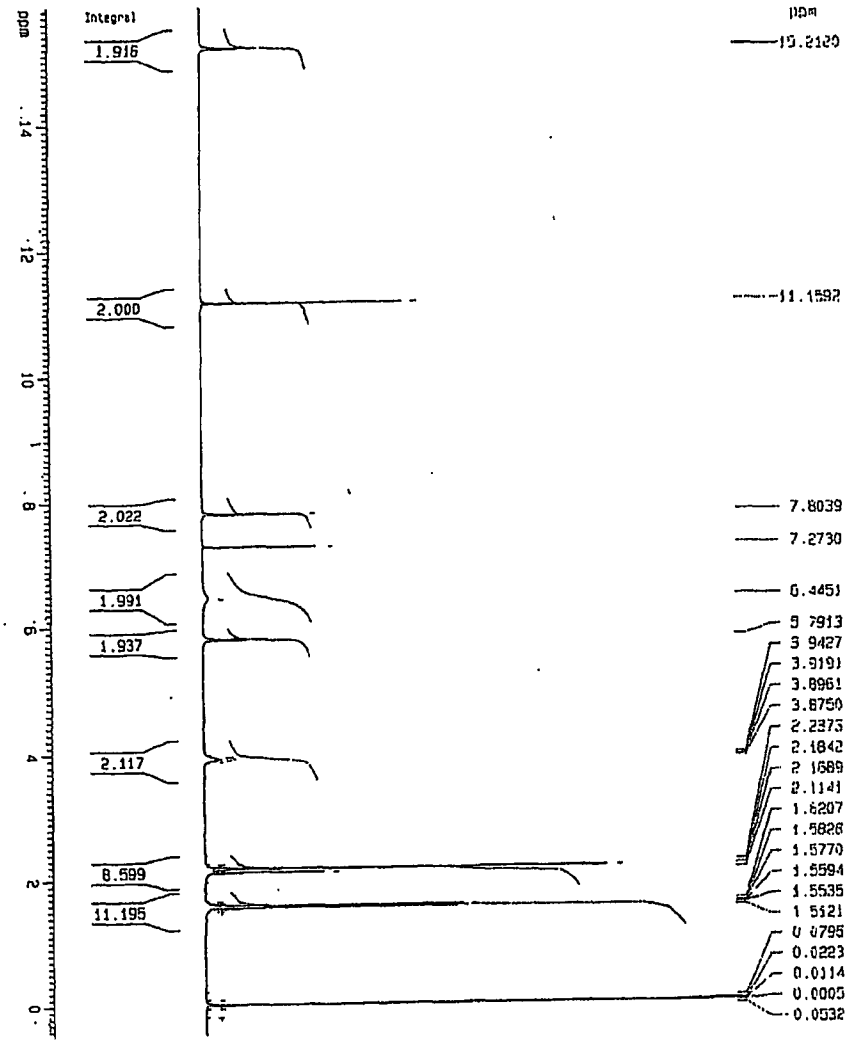
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FIG. 1

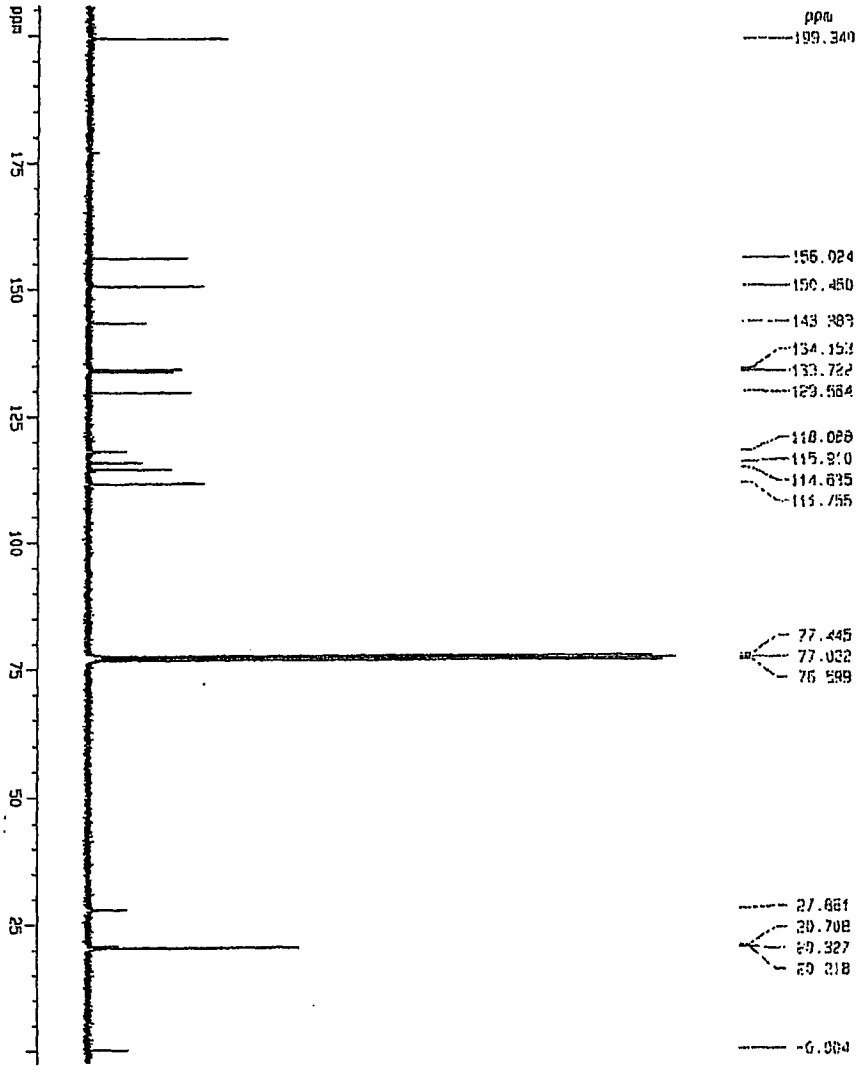
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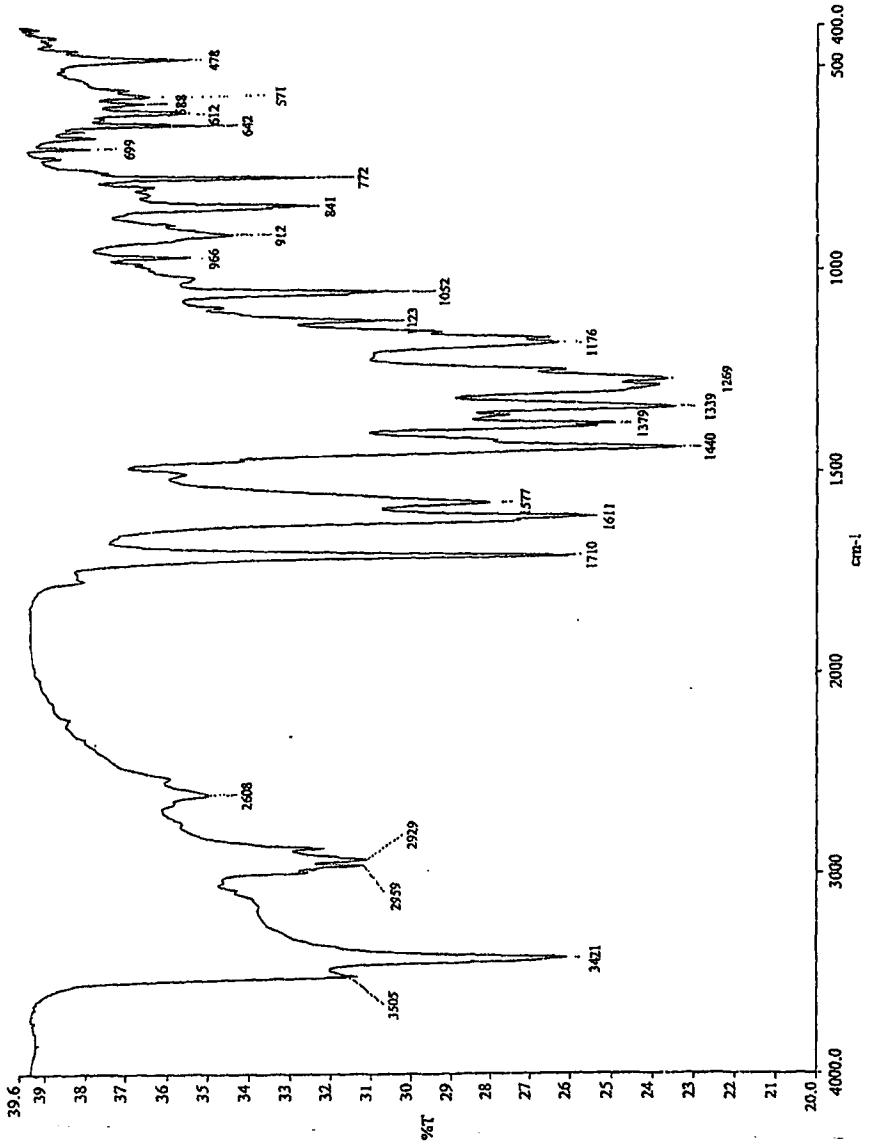
FIG. 2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3505
%T
cm-1
4000.0
3000
2000
1500
1000
500
400.0
39.6
39
38
37
36
35
34
33
32
31
30
29
28
27
26
25
24
23
22
21
20.0

FIG. 3

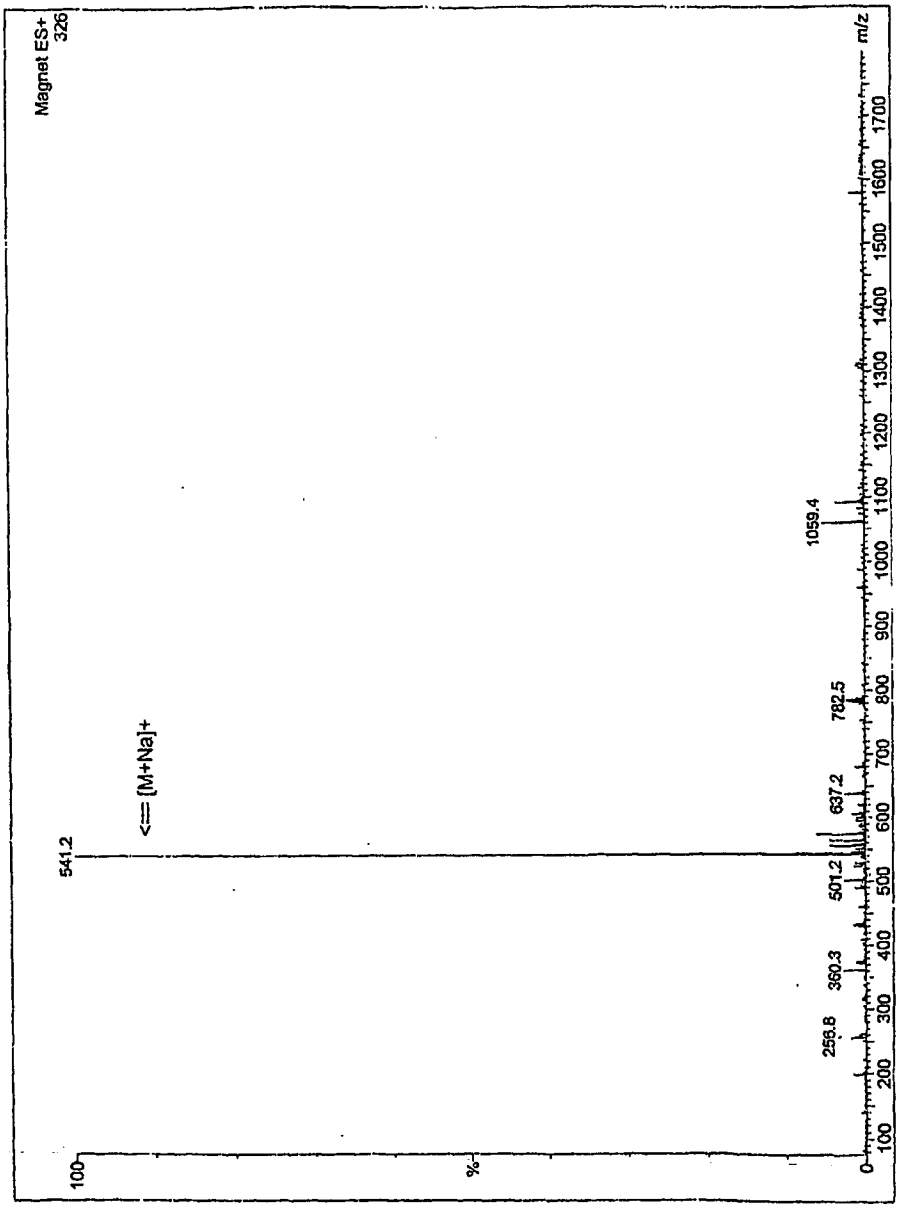

FIG. 4

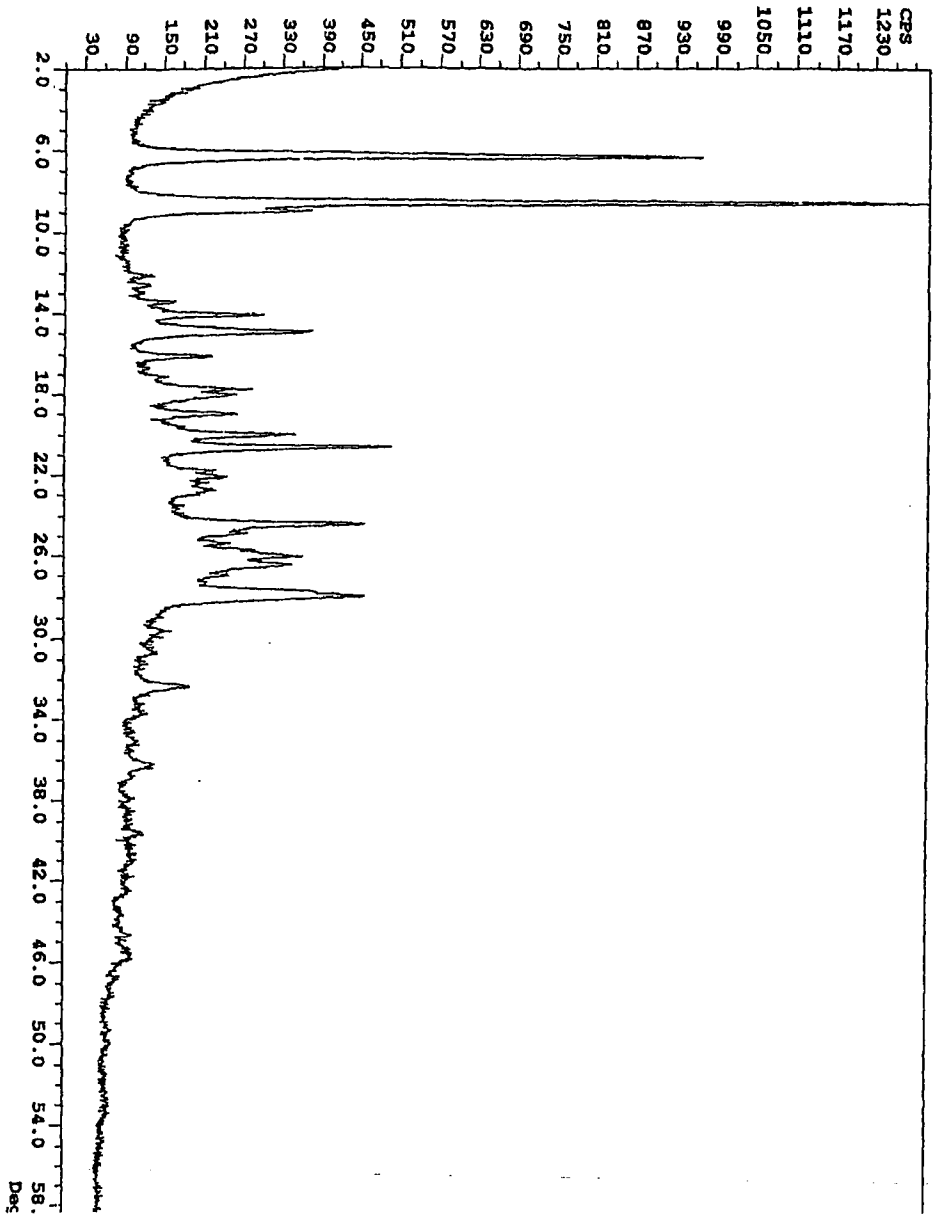
CPS
30
90
150
210
270
330
390
450
510
570
630
690
750
810
870
930
990
1050
1110
1170
1230
2.0
6.0
10.0
14.0
18.0
22.0
26.0
30.0
34.0
38.0
42.0
46.0
50.0
54.0
58.
Deg

FIG. 5

INTERNATIONAL SEARCH REPORT

International ap
PCT/US05/09673

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : A61K 31/11, 35/78
US CL : 514/700, 682; 530/377
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 514/700, 682; 530/377

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2003/0008924 (WANG et al.) 09 January 2003 (09.01.2003) page 2, paragraph 0017, 0018, 0019, 0025	1-28
Y	US 6,114,397 A (FLACK et al.) 05 September 2000 (05.09.2000) see abstract, column 1, line 45 to column 2, line 8, column 3, lines 1-11.	1-28
Y	US 6,696,484 B2 (LIAO et al.) 24 February 2004 (24.02.2004) see abstract, see claim 4, column 3, lines 43-49, column 10, lines 30-49.	1-28

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
27 June 2005 (27.06.2005)

Date of mailing of the international search report
11 JUL 2005

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Form PCT/ISA/210 (second sheet) (January 2004)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US05/09673

Continuation of B. FIELDS SEARCHED Item 3:
WEST 2.2.2, search terms: gossypol, co$1crystal apoptosis formic acid or acetic acid or propionic acid or $2butyric acid or $2pentanoic acid or $2hexanoic acid or $2heptanoic acid or $2octanoic acid or acrylic acid or succinic acid or fumaric acid or malic acid or tartaric acid or citric acid or lactic acid or benzoic acid and $4gossypol.clm and methanesulfonic acid or ethanesulfonic acid or $2propanesulfonic acid or $2butanesulfonic acid or $2pentanesulfonic acid or $2hexanesulfonic acid or $2octanesulfonic acid or benzenesulfonic acid and crystal ,